Geographical Variation in the Penetrance of CDKN2A Mutations for Melanoma

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Background: **Germline mutations in the CDKN2A gene, which encodes two proteins (p16INK4A and p14ARF), are the most common cause of inherited susceptibility to melanoma. We examined the penetrance of such mutations using data from eight groups from Europe, Australia and the United States that are part of The Melanoma Genetics Consortium.** ***Methods:*** **We analyzed 80 families with documented CDKN2A mutations and multiple cases of cutaneous melanoma. We modeled penetrance for melanoma using a logistic regression model incorporating survival analysis. Hypothesis testing was based on likelihood ratio tests. Covariates included gender, alterations in p14ARF protein, and population melanoma incidence rates. All statistical tests were two-sided.** ***Results:*** **The 80 analyzed families contained 402 melanoma patients, 320 of whom were tested for mutations and 291 were mutation carriers. We also tested 713 unaffected family members for mutations and 194 were carriers. Overall, CDKN2A mutation penetrance was estimated to be 0.30 (95% confidence interval (CI) = 0.12 to 0.62) by age 50 years and 0.67 (95% CI = 0.31 to 0.96) by age 80 years. Penetrance was not statistically significantly modified by gender or by whether the CDKN2A mutation altered p14ARF protein. However, there was a statistically significant effect of residing in a location with a high population incidence rate of melanoma (*P *= .003). By age 50 years CDKN2A mutation penetrance reached 0.13 in Europe, 0.50 in the United States, and 0.32 in Australia; by age 80 years it was 0.58 in Europe, 0.76 in the United States, and 0.91 in Australia.** ***Conclusions:*** **This study, which gives the most informed estimates of CDKN2A mutation penetrance available, indicates that the penetrance varies with melanoma population incidence rates. Thus, the same factors that affect population incidence of melanoma may also mediate CDKN2A penetrance. [J Natl Cancer Inst 2002;94:894–903]**

INTRODUCTION

Autosomal dominant inheritance associated with multiple cases of melanoma within a family has been widely recognized. In addition, susceptibility to melanoma is associated with an increased incidence of nevi or dysplastic nevi in some families *(1–4)*. Linkage studies, cytogenetic studies, and loss-of-heterozygosity studies have contributed to the localization of a melanoma susceptibility gene to chromosome 9p21 *(5–10)* and to the subsequent cloning of CDKN2A, the first identified melanoma susceptibility gene *(11,12)*. The CDKN2A region of chromosome 9p21 encodes two distinct proteins translated in alternate reading frames (ARFs) from alternatively spliced transcripts. The alpha transcript, which comprises exons 1α, 2, and 3, encodes a low–molecular weight protein, p16INK4A. The p16INK4A protein binds to the cyclin-dependent kinases, CDK4 and CDK6, inhibiting their association with Cyclin D, thereby preventing the formation of CDK/Cyclin D complexes *(13)*. These complexes phosphorylate the retinoblastoma protein, allowing the cell to progress through the G1 cell cycle checkpoint *(13,14)*. Thus, p16INK4A acts as a tumor suppressor and negatively regulates cell growth by arresting cells in the G1 phase. The smaller beta transcript, which comprises exons 1β and 2, encodes the alternative protein product, p14ARF, which acts via the p53 pathway to induce cell cycle arrest or apoptosis *(15,16)*.

Germline CDKN2A mutations have been identified in melanoma-prone families from Australia, Europe, and North America. Most CDKN2A mutations described to date are missense mutations scattered throughout exons 1α and 2 *(17)*. Overall, CDKN2A mutations have been observed in approximately 20% (range <5% to >50% in individual studies) of tested melanoma families *(18,19)*. In addition, linkage studies suggest that approximately one half of families with three or more cases of melanoma show evidence of linkage to the 9p21 region *(20–23)*.

Epidemiologic studies suggest that exposure to sunlight is the major environmental risk factor associated with melanoma, al-

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See "Notes" following "References."

though the exposure–response relationship appears complex [for review, see *(24)*]. The major host factors associated with melanoma are increased numbers of melanocytic nevi, both clinically banal and atypical (dysplastic) *(24,25).* Other host factors associated with melanoma include hair color, eye color, extent of freckling, and skin type *(26,27).* Differences in the amount of exposure to ultraviolet (UV) radiation by geographic latitude and variation in host characteristics may contribute to the wide geographic variation in melanoma incidence rates. Within Australia, annual age-standardized melanoma incidence rates per 100 000 people from 1992 through 1996 varied with geographic latitude, from 63 for males and 46 for females in Queensland to 33 for males and 28 for females in Victoria *(28).* For the U.S. Surveillance, Epidemiology and End Results Program of the National Cancer Institute (SEER),[1] the overall annual age-standardized incidence rate per 100 000 people was 19.6 for males and 12.6 for females in 1994 *(29);* the U.K. rates were 3.9 for males and 6.8 for females from 1983 through 1987 *(30).* Rates within Western Europe are generally similar, except in Scandinavian countries, where the rates are more in line with those in the United States *(30).*

The Melanoma Genetics Consortium was founded in 1997 to address issues related to inherited melanoma susceptibility. One of its first projects has been the examination of the penetrance of CDKN2A mutations for melanoma. All of the groups participating in this consortium have been involved in identifying families with multiple cases of melanoma and in investigating CDKN2A mutations. The interest in evaluating CDKN2A penetrance arises for a number of reasons, most notably that the clinical application of this information is being discussed *(17).* In addition, studies of melanoma etiology will be enhanced by clearer documentation of the contribution of the CDKN2A gene to melanoma incidence.

The goals of this study were, therefore, to determine the penetrance of CDKN2A mutations and to test whether any of three covariates—gender, the effect of the CDKN2A mutation on the p14ARF protein, or the population melanoma incidence rate relevant to the geographic location of residence of each family—influences penetrance. Families are identified for such genetic studies because their family history of melanoma is sufficiently strong that it is brought to the attention of the medical profession; however, not all families with a similar extent of family history will be identified. As with other disease susceptibility genes identified through family studies, the estimation of penetrance is, therefore, not straightforward because of the difficulty in defining the particular reason that each family is identified (termed "ascertainment"). Families with large numbers of affected individuals are presumably more likely to be identified than families with only a few affected individuals; failure to correctly allow for this factor in the calculations would overestimate the risk of melanoma among mutation carriers within such families. To overcome the uncertainty of the precise reason that a specific family is identified, the statistical approach of preference is to perform the penetrance calculations under the assumption that the family was identified because of the exact configuration of affected persons actually observed.

Subjects and Methods

Participating Groups

Eight groups from the Melanoma Genetics Consortium contributed information concerning the genealogical structure of families with multiple cases of melanoma and a CDKN2A mutation, the melanoma status of family members, the CDKN2A mutation carrier status of family members, the age of onset for those family members with melanoma, the age at which each individual was last examined (or known to be unaffected) or the age at death for family members, and the CDKN2A mutation segregating in each family to these analyses (Table 1).

Family ascertainment and sampling. For all groups, written informed consent was obtained from the subjects before participation in the study under an Institutional Review Board–approved protocol. The precise methods used in the ascertainment of families with multiple cases of melanoma differed between groups. Except as indicated below, no restrictions were placed on the degree of genetic relationship of case patients for ascertainment purposes. Following agreement of the case patients to participate, unaffected relatives were also contacted and asked to participate in the study. Family members were asked to give a blood sample for DNA analysis following informed consent. The primary focus for collection of blood samples involved first-degree relatives of family members with a diagnosis of melanoma and obligate CDKN2A mutation carrier relatives, although more genetically distant relatives were also contacted if feasible. Many of the groups also routinely performed a skin examination of family members. Each participating center in this research identified families from a wide geographic area in view of the infrequency of such families. The procedures for identifying families within each center follow.

Families submitted from the Queensland Institute of Medical Research, Brisbane, Australia: To be eligible for participation, families needed to have had at least three living family members with melanoma willing to donate blood for linkage and DNA analysis *(31,32).* Ten of the 15 mutation-positive families were ascertained in two population-based studies of melanoma *(33,34).* The remaining five families were referred by local surgeons in an *ad hoc* manner.

Families submitted from Westmead Institute for Cancer Research, Sydney, Australia: Individuals with a self-reported family history of melanoma were identified through several large participating clinics, notably the Sydney Melanoma Unit, Sydney, and the Victorian Melanoma Service, Melbourne, and by other referring clinicians throughout southeastern Australia since 1985. The cancer history of each case patient and his or her relatives was confirmed from medical records and extended as far as possible, essentially until no cases of melanoma were reported within two degrees of relationship to the last confirmed case ascertained. For the families reported in this study, an attempt was made to trace back the CDKN2A mutation as far as possible and then to identify all descendents of the most ancestral couple thought to carry the CDKN2A mutation. The disease status of all families reported here was updated within the year before this analysis. The collection of these families and the precise mutations identified is described in more detail elsewhere *(35–38).* Blood samples have been routinely collected for DNA analysis, but phenotypic examination of skin has not routinely been performed.

Families submitted from Cancer Research UK, Leeds, U.K.: Melanoma families were identified by contacting dermatologists, surgeons, and clinical geneticists throughout the United Kingdom and outlining the criteria for participation; these criteria included that there be at least two cases of melanoma in the family and that at least one living case patient be willing to

Table 1. The families from the Melanoma Genetics Consortium contributing to this analysis, the melanoma incidence in their geographic location *(30),* and the summary of families included in the analysis

Consortium groups	Baseline incidence of melanoma			Case patients				Unaffected subjects		
Geographic region and location	Cumulative incidence to age 75 males (%)*	Cumulative incidence to age 75 females (%)*	No. of families	No. of melanoma case patients	No. of melanoma case patients tested for mutation	No. (percentage) of tested case patients with mutation	Mean age at diagnosis (y) of mutation carriers (range)	No. of unaffected subjects tested for mutation	No. (percentage) of tested unaffected subjects with mutation	Mean current age (y) of unaffected carriers (range)
Australia										
Queensland Institute of Medical Research, Brisbane	2.82†	2.38†	15	98	81	71 (88%)	38.6 (12–86)	60	12 (20%)	51.6 (34–89)
Westmead Institute of Cancer Research, Sydney	2.82†	2.38†	13	63	49	44 (90%)	35.3 (15–61)	81	25 (31%)	44.7 (15–77)
USA										
National Cancer Institute, Bethesda	1.16‡	0.86‡	15	95	77	72 (94%)	35.0 (14–68)	250	54 (22%)	33.0 (5–80)
Europe										
Karolinska Institute, Sweden	1.04‡	0.97‡	6	23	22	19 (86%)	45.2 (22–73)	32	13 (41%)	50.1 (30–75)
University of Genova, Italy	0.52§	0.43§	9	26	10	10 (100%)	38.1 (23–60)	24	7 (29%)	37.4 (14–65)
Leiden University, Netherlands	0.34‖	0.52‖	6	35	35	31 (89%)	37.1 (16–72)	139	38 (27%)	42.2 (25–76)
Cancer Research UK, Leeds, U.K.	0.30‡	0.51‡	5	24	18	17 (94%)	40.1 (20–79)	57	23 (40%)	36.3 (16–70)
Institut Gustav Roussy, France	0.07–0.57¶	0.34–0.67¶	11	38	28	27 (96%)	38.7 (21–69)	70	22 (31%)	33.2 (4–68)
TOTAL			80	402	320	291 (91%)	37.5 (12–86)	713	194 (27%)	39.2 (4–89)

*Cumulative incidence rates are for the time period 1983–1987 *(30).*

†Cumulative incidence rates taken from the tables for the New South Wales registry.

‡National figures for 1983–1987 (SEER for the United States).

§Figures from the Genoa, Italy, Registry.

‖Figures from the Maastricht, The Netherlands, Registry.

¶Range of figures for cancer registries in France.

provide a blood sample for DNA analysis. Each family was visited by a dermatologist involved in the research (usually Dr. J. Newton Bishop). A blood sample was taken and a skin examination performed. Family members with melanoma and those without melanoma were examined. Attempts have been made to identify all branches of the families in this study independent of the presence or absence of melanoma. More details about the families can be found elsewhere *(36,39,40).*

Families submitted from Institut Gustave Roussy, Villejuif, France: Melanoma families were identified by contacting dermatologists and geneticists throughout a French clinician network co-coordinated at the Institut Gustave Roussy. The criteria for inclusion in the study included the presence of at least two cases of melanoma in the family, confirmed by pathological reports or medical records. A blood sample was taken for DNA analysis and a skin examination was performed on family members with melanoma and on those without melanoma. The families are described in more detail elsewhere *(18).*

Families submitted from University of Genova, Genova, Italy: Families were ascertained predominantly through patients presenting to Italian medical genetics services after their having been identified by dermatologists or oncologists as having multiple family members with melanoma; the dermatologists and oncologists had been informed of the activity of the melanoma research group at national conferences. The selection criteria were for families to have at least two cases of melanoma and at least one living case patient willing to provide a blood sample for DNA analysis. For expediency, some family members were visited by dermatologists or surgeons not involved in the research program. Blood samples have been taken for DNA analysis and the skin examined for as many family members as possible. The families are described in more detail elsewhere *(41).*

Families submitted from Leiden University Medical Center, The Netherlands: The identification of Dutch families with multiple cases of melanoma began with a cohort of melanoma patients attending the surgical oncology department of the Leiden University Medical Center, Leiden, The Netherlands, between 1982 and 1984. This cohort of case patients, which were being followed up, and more recently diagnosed case patients were interviewed on two separate occasions about the presence of melanoma in their relatives. In the case of a positive response of any relative with a confirmed diagnosis of melanoma, all first-degree and second-degree relatives of the initial case patient were ascertained for inclusion in this study. All family members were interviewed about their family and personal medical history, had their skin examined, and had a blood sample taken for DNA analysis by the same dermatologist (Dr. W. Bergman). The families are described in more detail elsewhere *(42,43).*

Families submitted from Karolinska Hospital, Stockholm, Sweden: Melanoma families were identified in a national program aimed at detecting and monitoring melanoma families that was started by the Swedish Melanoma Study Group in 1987. All

case patients with cutaneous melanoma were questioned with respect to their family history of melanoma, and the diagnosis of melanoma in other family members was verified by histopathology reports. Criteria for inclusion of the families in the study was that they had to have two or more family members with verified cutaneous melanoma. Family members including all case patients and unaffected relatives were followed clinically at specialized outpatient clinics at several centers in all health care regions of Sweden. The families are described in more detail elsewhere *(44–46).*

Families submitted by the Genetic Epidemiology Branch, National Cancer Institute, Bethesda, USA. Melanoma families were referred to the National Cancer Institute by healthcare professionals or through self-referral. Families were eligible for inclusion in the study if there were at least two living first-degree relatives with invasive melanoma. Clinical examination of family members and spouses included complete skin examination (by Dr. M A Tucker), routine medical history, and a blood sample for DNA analysis. Both affected case patients and unaffected relatives were examined. The families are described in more detail elsewhere *(11,19).*

Confirmation of diagnoses. Melanoma cases were verified as far as possible from cancer registries, pathology laboratories, or clinical records. We have not summarized the extent of verification, as the procedures differ by center and family; however, every effort was made to confirm diagnoses, and only those family members with convincing evidence (i.e., pathology confirmation or medical record or, in a few exceptional circumstances, death certificate) were included as affected in these analyses.

Inclusion criteria for families. In an attempt to introduce more consistency among the varied ascertainment approaches of the participating groups, families were only included in the analysis if each had at least two cases of cutaneous malignant melanoma in first-degree relatives and a proven germline mutation in CDKN2A present in at least one family member. Several groups had identified families with CDKN2A mutations that did not satisfy this relatedness criterion. These few families were not included in this analysis. CDKN2A mutations were considered as causal if they cosegregated with melanoma in multiple-case families or if a binding assay of p16INK4A activity confirmed a functionally deficient p16INK4A protein with impaired binding to one or more cyclin-dependent kinases *(47).*

Statistical analysis. Family data were sent in a standard format to the Analysis Team (D. T. Bishop, F. Demenais, and A. Goldstein). The data included the genetic relationships of all individuals within each family, a phenotypic record for each family member indicating melanoma status and age at first diagnosis for those individuals diagnosed with melanoma, age at examination or age last known to be unaffected with melanoma for unaffected family members and demographic information (birth year, age at death or current age, gender), and CDKN2A mutation status of all tested individuals. For each family, the country of residence was recorded, as were the CDKN2A mutation segregating in the family and whether this mutation affected the reading frame of the p14ARF protein or the p14ARF protein sequence. These family data were stored in a common database (held by D. T. Bishop). For this analysis of penetrance, only individuals with a diagnosis of cutaneous malignant melanoma were considered to be "affected." Family members with a diagnosis of cancer other than melanoma (and without a diagnosis of melanoma) were considered as "unaffected with melanoma" for the penetrance analysis. Pancreatic cancer has been reported in some melanoma families to be observed at increased frequency, but for the purposes of this study such diagnoses were ignored *(19,41).* The penetrance estimates, therefore, relate to the risk of developing melanoma rather than to the risk of developing any cancer.

The penetrance of the CDKN2A gene mutation was estimated with a logistic regression model *(48)* extended to take into account variable age at diagnosis of disease *(49)* and linked marker loci *(50).* The regression model is formally known as a Class D regressive model, which in its generality allows the construction of patterns of correlations within families to include both genetic and nongenetic factors. For this analysis, in which we assume that the CDKN2A mutation is the only cause for the number of cases of melanoma within the family, the regression model is constructed by specifying a regression relationship between each individual's phenotype (i.e., affected or unaffected with melanoma) and a set of explanatory variables, including the individual's CDKN2A genotype and covariates such as the individual's age, gender, and geographic location of residence. Under this model, the probability of observing a family with a particular configuration of affected and unaffected individuals is written as the product of the probability of the vector of genotypes at the disease-causing locus multiplied by the penetrance function summed over all unobserved disease genotypes in family members. In this analysis, the summation is only over individuals with an unknown CDKN2A genotype. For those individuals, the probability of unobserved genotypes is expressed in terms of the frequency of the CDKN2A mutation in the general population if that individual has no ancestors in the pedigrees (founders of the pedigrees and spouses) and in terms of Mendelian probabilities if that individual does have ancestors recorded within the family. The frequency of the CDKN2A mutation was set at 0.0001 (Demenais, F: unpublished data), which is believed to be the approximate frequency of all CDKN2A mutations combined in the general population; that is, the source of the spouses of case patients.

The penetrance function (probability of the disease phenotype, Y, given the vector of genotypes, g, at the CDKN2A locus and covariates, X) over n individuals in the family, is decomposed into a product of penetrance functions for each individual i:

$$P(Y|g, X) = \prod_{i=1}^{n} (P_i|g_i, X_i),$$

where g_i is the ith individual's genotype and X_i is the vector of covariates for i. Survival analysis concepts were introduced to take into account a censored age at diagnosis of melanoma *(48).* Age at diagnosis is regarded as a failure time, and age at examination (for unaffected family members) as a censored failure time, where the scale for measuring time is age. We began counting the follow-up time at 15 years of age because experience with this and other unpublished studies (Demenais F, Goldstein A: unpublished observation) suggests that the fit of the model is improved with this offset (in fact, in this dataset, there were two individuals with age at diagnosis under the age of 15 years, so we recoded their ages of diagnosis to 15 years). The period of follow-up was taken from 15 years of age to age at diagnosis for affected case patients, age at examination for un-

affected subjects (or affected case patients with unknown age at diagnosis), or age at death for deceased subjects (there were three individuals with convincing diagnoses of melanoma in the dataset but for which ages at diagnosis were not available; for these individuals, their age at examination were taken as their age at diagnosis). This follow-up period was partitioned into K mutually exclusive intervals of one year each ($1 \ldots K$). In each interval, we computed the hazard function, $\lambda(k)$, which is the probability of being affected in the kth interval given not being affected before. The penetrance function is then derived from the hazard function $\lambda(k)$. For affected cases, the penetrance function is a density function evaluated at that individual's actual age of diagnosis (k):

$$f(k) = \lambda(k) \prod_{h=1}^{k-1} [1 - \lambda(h)].$$

For unaffected family members, the penetrance function is the probability of being unaffected at all ages up to the current age (k):

$$S(k) = \prod_{h=1}^{k} [1 - \lambda(h)].$$

For the three case patients with an unknown age at diagnosis but a known age at examination (k), the penetrance becomes $F(k) = 1 - S(k)$. The penetrance function is defined as one for individuals with unknown disease status. The hazard function $\lambda_i(k)$, for the ith individual in the kth interval, is then a logistic function: $\lambda_i(k) = \exp\{\theta_i(k)\}/(1 + \exp\{\theta_i(k)\})$, where $\theta_i(k)$, the logit of the hazard function, is

$$\theta_i(k) = \alpha_{gi} + \beta_{gi} X_i(k) + u_{gi}(k).$$

The parameter α_g is the genotype-specific baseline parameter; β_g is the row vector of genotype-specific regression coefficients for covariates $X(k)$ that can be time dependent; $u_g(k)$ is a function of k that represents the variation of the logit of the hazard function with time and can be genotype dependent. Because the disease-associated CDKN2A mutations are assumed to have a dominant mode of inheritance, the model includes two genotype-specific baseline risks: α_{Aa} for mutation carriers ($\alpha_{AA} = \alpha_{Aa}$) and α_{aa} for mutation noncarriers. The hazard function can be assumed to be constant over time ($u_g[k] = 0$) or varying with time using different parametric functions of k; k increases from 1 in the first interval (corresponding here to 15 years of age for each individual) to K, K being equal to the individuals' age at diagnosis (or age at examination) minus 15 years. We found that the function $u_g(k) = \delta_g \ln(k)$ fit the data better (Demenais F, Goldstein A: unpublished observation) than a polynomial function of k (linear, quadratic or cubic). Moreover, the variation of the hazard function with time did not differ in mutation carriers and noncarriers. The function, $u(k) = \delta \ln(k)$ was then used in all analyses.

The above calculation produces the probability of a particular configuration of individuals with defined melanoma status, ages (at diagnosis and at last examination), and CDKN2A mutation status (termed the "likelihood"). To estimate penetrance, we needed to take into account that we did not ascertain these families by chance; rather, we found them because of the large number of melanoma cases within each family. The likelihood on which to base the penetrance estimation is, therefore, the likelihood as described above conditional on the disease phenotypes (affected or unaffected with melanoma) in all individuals. This conditional likelihood represents an assumption-free method of ascertainment and is known to yield unbiased parameter estimates *(51).* Parameter estimation and tests of hypotheses were carried out using maximum-likelihood methods as implemented in the computer program REGRESS *(52),* which performs such likelihood calculations for family data. The analysis (performed in REGRESS) considered two distinct loci, a disease locus and a marker locus (CDKN2A) with equal allele frequencies but without linkage disequilibrium and assuming complete linkage between these two loci in the numerator of the likelihood and no linkage in the denominator. The effects of covariates on the penetrance function were tested by a likelihood-ratio test that compares a submodel where the regression coefficient, β, of a given covariate is set to zero with a model where it is estimated.

The covariates included in the model were gender, the effect of the CDKN2A mutation on the p14ARF protein, and geographic location. Gender was coded 0 for females and 1 for males. The p14ARF variable was coded 0 if the CDKN2A mutation was predicted not to affect the p14ARF coding sequence and 1 otherwise. We adopted two approaches to the examination of the effect of geographic location. The geographically separated groups of families represent a wide range of melanoma population incidence rates. Because of the limited statistical information from each group, we dichotomized the geographic locations of each group into low-incidence countries (European countries except Sweden) and high-incidence countries (Sweden, the United States, and Australia). With the comparison of low-incidence and high-incidence countries, we could examine the effect of baseline melanoma incidence rates on CDKN2A mutation penetrance. In addition, to relate the penetrance of the CDKN2A gene mutation to more readily interpretable geographic and population units, we estimated the penetrance separately in three geographic areas: Europe (excluding Sweden), the United States, and Australia. The only problem with respect to this categorization was that, on the basis of baseline melanoma incidence rates, Sweden should not be pooled with Europe for the geographic comparisons of CDKN2A gene mutation penetrance. However, there were too few Swedish families to allow separate estimation of the penetrance. In addition, although baseline incidence rates for melanoma are similar between the United States and Sweden, substantial differences in major melanoma risk factors, such as UV exposure, tanning ability, skin complexion, and eye and hair color precluded conducting a meaningful analysis of penetrance when combining families from the United States and Sweden. Hence, for the analyses by geographic region, the Swedish families were excluded.

We varied the CDKN2A allele frequency from the value used in the reported analyses (0.0001) and have found that the estimates of penetrance are robust to reasonable changes in this value (data not shown) so that only the estimates for the one allele frequency are included here.

Laboratory Methods

CDKN2A gene mutations were identified either as a one-stage process involving direct sequencing of CDKN2A using the PCR technique described previously *(39)* or as a two-stage process involving an initial screen for heterozygous bases with single-stranded conformational polymorphism followed by sequencing of PCR products identified as containing heterozygos-

ity [as, for instance, in *(34)*] based on PCR products as described previously *(11,12)*.

RESULTS

Eight groups from the Melanoma Genetics Consortium contributed family data information to this analysis of CDKN2A mutation penetrance (Table 1). A total of 80 families met the criteria for inclusion (Table 1). These families included 402 reported cases of melanoma (an average of 5.0 per family). Thirteen families had two cases of melanoma, 17 families had three cases, 30 families had from four to six cases, 15 families had from seven to 10 cases, and five families had more than 10 cases (to a maximum of 17 cases in one family). Eighty percent of the reported melanoma patients were tested for the CDKN2A gene mutation in their family, and the majority (91%) carried the family mutation. The average age at diagnosis of mutation carriers did not vary dramatically among the centers, ranging from a low of 35.0 years in the United States to a high of 45.2 years in Sweden. The overall range in age at diagnosis of mutation carriers was from 12 years to 86 years (Table 1). The majority of patients were either first-degree or second-degree relatives of other patients; only nine of the patients (2.2% of all patients) were a third-degree or higher relative of the most closely related melanoma patient.

The penetrance estimation derives most information from the carrier status (i.e., mutation carrier or noncarrier) of unaffected relatives of melanoma case patients and especially from the elderly unaffected relatives. A large number of elderly unaffected mutation carriers would indicate a lower penetrance of a CDKN2A mutation than would a paucity of such individuals. Among the unaffected family members, 713 were mutation tested, and 194 of them carried the CDKN2A mutation. The current average ages of the unaffected family members who carried the CDKN2A mutation ranged from 33.0 years in the United States to 51.6 years in Queensland, Australia (Table 1). Overall, the mean current age of unaffected noncarriers of the family mutation was 5.6 years older than the mean current age of unaffected carriers (data not shown).

Table 2 shows the CDKN2A mutations in the study families in the standard convention for presenting mutations *(53)* together with the number of families carrying each mutation. Of the 37 distinct mutations, only nine occurred in more than one

Table 2. The CDKN2A mutations identified in consortium families, their location and their effect on p14ARF *(53)*.

Location of mutation	CDKN2A nucleotide change*	Effect on CDKN2A protein sequence	Effect on $p14^{ARF}$ protein sequence†	No. of families carrying mutation
Exon				
1α	-34G>T	No amino acid change, false ATG	N/A	1
1α	9-32del24	In-frame deletion (3-10del8)	N/A	1
1α	9-32dup24	In-frame insertion (1-8dup8)	N/A	4
1α	44G>A	Nonsense (Trp15Stop)	N/A	1
1α	46delC	Frameshift 16-24, Stop 25	N/A	1
1α	47T>G	Missense (Leu16Arg)	N/A	1
1α	47T>C	Missense (Leu16Pro)	N/A	1
1α	68G>A	Missense (Gly23Asp)	N/A	1
1α	71G>C	Missense (Arg24Pro)	N/A	4
1α	88delG	Frameshift 30-51, Stop 52	N/A	1
1α	95T>C	Missense (Leu32Pro)	N/A	2
1α	104G>C	Missense (Gly35Ala)	N/A	1
1α	106G>C	Missense (Ala36Pro)	N/A	1
1α	143C>T	Missense (Pro48Leu)	N/A	1
1α	146T>G	Missense (Ile49Ser)	N/A	1
1α	149A>G	Missense (Gln50Arg)	N/A	1
2	159G>C	Missense (Met53Ile)	Missense (Asp68His)	8
2	167G>T	Missense (Ser56Ile)	Missense (Gln70His)	1
2	167-197del31	Frameshift 67-144, Stop 145	Fusion p14ARF 1-70, p16INK4A 70-156	1
2	172C>T	Nonsense (Arg58Stop)	Missense (Pro72Leu)	1
2	185T>C	Missense (Leu62Pro)	Silent (Ala76Ala)	1
2	199G>A	Missense (Gly67Ser)	Missense (Arg81Gln)	1
2	202-3GC>TT	Missense (Ala68Leu)	Missense (Arg82Leu)	1
2	212A>G	Missense (Asn71Ser)	Silent (Gln85Gln)	2
2	213C>A	Missense (Asn71Lys)	Missense (Leu86Met)	1
2	225-243del19	Frameshift 76-138, Stop 139	Fusion p14ARF 1-90, p16INK4A 82-156	7
2	240-253del14	Fusion p16INK4A 1-80, p14ARF 100-133	Frameshift 96-155, Stop 156	1
2	260G>C	Missense (Arg87Pro)	Silent (Pro101Pro)	1
2	290T>G	Missense (Leu97Arg)	Silent (Pro113Pro)	1
2	301G>T	Missense (Gly101Trp)	Missense (Arg115Leu)	16
2	322G>A	Missense (Asp108Asn)	Missense (Arg122Gln)	1
2	334C>G	Missense (Arg112Gly)	Missense (Pro126Arg)	1
2	337-338insGTC	In-frame insertion (112-113insArg)	In frame insertion (127-128insSer)	5
2	352G>A	Missense (Ala118Thr)	Missense (Gly132Asp)	1
2	373G>C	Missense (Asp125His)	N/A	1
2	377T>A	Missense (Val126Asp)	N/A	4
Intron 2	IVS2+1G>T	Deletion, insertion (153Asp155ProDel,Ins ValGlu)	N/A	1

*Nucleotides are numbered from the first A of the initiation codon of p16INK4A in the standard nomenclature for mutations employed *(53)*.

†N/A = Not applicable (no change in coding sequence).

family. Of these mutations, three were founder mutations within the respective populations: 225-243del19 mutation from the Netherlands *(43)* which was seen in seven families, 112-113insArg mutation from Sweden (in five families) *(54),* and Gly101Trp mutation from Southeastern Europe (in 16 families) *(55,56).* Overall, approximately half of the mutations were in exon 1α and half were in exon 2. Table 2 also indicates the effect of the CDKN2A mutation on the reading frame of p14ARF and the p14ARF protein coding sequence. Mutations in exon 1α will have no effect on p14ARF protein, whereas some mutations in exon 2 will have an effect on p14ARF. No mutations were identified that modified the p14ARF protein and not CDKN2A.

Estimates of the parameters of the regressive models and tests of hypotheses are shown in Table 3. The penetrance of CDKN2A was not statistically significantly modified by gender ($P = .70$) or by whether the CDKN2A mutation altered the p14ARF protein ($P = .28$). The logistic model (Table 3) suggested that CDKN2A mutations in males had 0.76 times the hazard rate of those mutations in females, whereas mutations that also affected p14ARF had a hazard rate 1.79 times that of mutations that did not affect p14ARF. However, there was a highly statistically significant effect of residing in a location with a high population incidence rate of melanoma ($P = .003$). The hazard function of melanoma in high-incidence countries (Australia, Sweden, and the United States) was 3.74 times that in low-incidence countries (European countries excluding Sweden). Comparison of Europe (including Sweden) versus the United States and Australia ($P<.001$) showed that the statistically significant difference in the hazard function was robust to the category to which the Swedish data were assigned (data not shown).

We next estimated the age-specific penetrance estimates of CDKN2A mutations by geographical region. The age-specific penetrance estimates of CDKN2A mutations from all geographic regions combined are shown in Figure 1. The penetrance of the CDKN2A mutations reached 0.30 (95% confidence interval [CI] = 0.12 to 0.62) by age 50 years and 0.67 (95% CI = 0.31 to 0.96) by age 80 years. Fig. 1 also presents the location-specific estimates of penetrance in mutation carriers for Europe, Australia, and the United States. Because of the higher baseline incidence rates of melanoma in Sweden, the Swedish sample was excluded from the penetrance assessment of the other European countries. The small numbers of available Swedish families also precluded a separate evaluation of penetrance. Fig. 1 shows that the penetrances of CDKN2A mutations were always lower in European countries than in the two other continents studied. Interestingly, there was a crossover in CDKN2A mutation penetrance at age 65 years between the United States and Australia, with penetrance being higher in the sample from the United States than in the Australian sample for individuals younger than 65 years of age and lower in the United States sample than the Australian sample thereafter. Penetrance of CDKN2A mutations by 50 years of age were 0.13 in European countries, 0.50 in the United States, and 0.32 in Australia, whereas the lifetime penetrance (by age 80 years) reached 0.58 in Europe, 0.76 in the United States, and 0.91 in Australia.

Discussion

Epidemiologic studies have shown that both genetic and environmental factors influence the risk of melanoma. CDKN2A germline mutations are the predominant recognized cause of inherited melanoma susceptibility, and exposure to UV radiation is the predominant environmental factor. This study addressed two issues, namely the collective penetrance of CDKN2A germline mutations and the possibility of modification of penetrance by environmental (as measured by baseline population incidence rates among the geographic locations) and other factors (gender and the effect of the CDKN2A mutation on the p14ARF coding). As expected, we found that penetrance of CDKN2A mutations across all locations studied was high, with an estimated 30% penetrance by age 50 years and 67% penetrance by age 80 years, although the confidence intervals were broad. Although these data provide the most definitive estimates of CDKN2A mutation penetrance to date, there is still considerable uncertainty about the precise risk of developing melanoma. Interestingly, however, the data were sufficient to show that one of the causes of uncertainty in the overall estimate of penetrance is the variation in

Table 3. Analysis of melanoma risk and CDKN2A mutation status using the logistic regression models that include age-specific penetrances for CDKN2A and covariates (gender, effect on the p14ARF protein, and population baseline incidence rates of melanoma [high incidence versus low incidence])*

Model	Allele frequency CDKN2A	α_{aa}†	α_{Aa}‡	δ§	β_{gender}‖	β_{p14ARF}¶	$\beta_{incidence}$#	−2ln likelihood	Test statistic χ^2	*P* value
1. No covariate	(0.0001)**	−11.17	−7.13	0.88				−192.82	—	—
2. Adding gender	(0.0001)	−10.82	−6.72	0.78	−0.27 (0.76)††			−192.97	0.15‡‡	.70
3. Adding p14ARF	(0.0001)	−11.97	−7.87	0.98		0.58 (1.79)††		−193.98	1.16	.28
4. Adding population incidence baseline	(0.0001)	−11.09	−7.01	0.56			1.32 (3.74)††	−201.55	8.73	.003

*Tests are based on liklehood ratio comparisons and are two-sided.

†Genotype-specific baseline parameter for noncarriers of CDKN2A mutation (α_{aa}).

‡Genotype-specific baseline parameter for carriers of CDKN2A mutation (α_{Aa}).

§δ is regression coefficient specifying the variation of the hazard function with time (on the logarithmic scale).

‖β_{gender} is the regression coefficient for males compared with females (referent group).

¶β_{p14ARF} compares CDKN2A mutations that affect the p14ARF sequence with those mutations that do not (referent group).

#$\beta_{incidence}$ is a binary variable indicating whether a family resides in countries with high base-line melanoma incidence rates (Australia, Sweden, and the United States) or in countries with low base-line incidence rates (France, Italy, Netherlands, and the United Kingdom) (referent group).

**Parameters in parentheses are fixed at the value in the parentheses. The allele frequency is fixed at 0.0001 (see Methods).

††The odds ratios of the hazard function for each of these covariates (OR = exp [β]) is shown, in parentheses, next to the regression coefficients.

‡‡Likelihood-ratio test of the absence of a given covariate effect (model 1) versus a model including the covariate (model 2, 3, or 4).

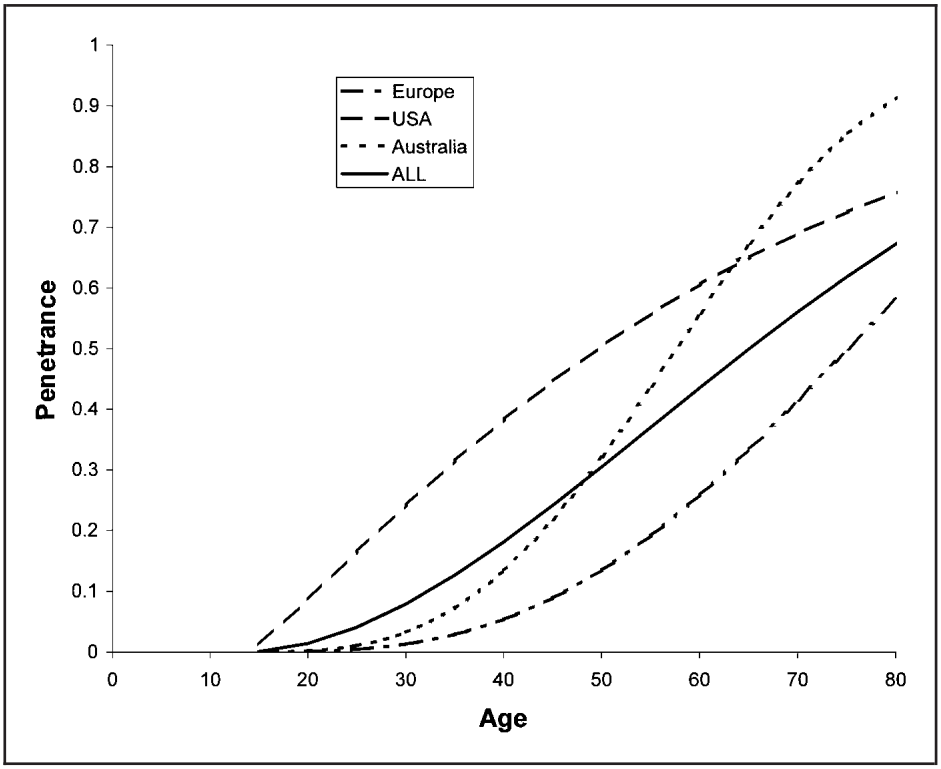

Fig. 1. Estimated age-specific penetrance estimates for CDKN2A mutations. Penetrances are shown for the total set of families in the study, assuming the same penetrance of mutations in all geographic locations (ALL); families living in Australia (Australia); families living in France, Italy, The Netherlands, or the United Kingdom (Europe); and families living in the United States (USA).

penetrance by baseline population incidence differences, with the hazard rate being an estimated 3.74 times higher in regions of the world with higher baseline incidence (Australia, the United States, and Sweden) than in regions with lower baseline incidence (Europe except Sweden). This proportionate increase in penetrance in CDKN2A mutation carriers between high-baseline and low-baseline incidence locations is similar in magnitude to the increase in risk of melanoma among the general population between high-incidence and low-incidence locations. Our data, therefore, are consistent with the same risk factors mediating risk to the same extent in CDKN2A mutation carriers as in mutation noncarriers—that is, with the lack of a gene-environment interaction. Analyses by geographic region (Australia, Europe, and the United States) showed the same overall effects of increasing penetrance with higher-baseline incidence rates but also contained some less readily interpretable features, such as evidence for a higher risk of melanoma in young mutation carriers in the United States as compared with young mutation carriers in Australia. We also conducted the analyses of geographic region using proportional hazards models without the logistic constraints included here and found essentially the same results (data not shown), indicating that this difference in the risk of melanoma between young mutation carriers in the United States and Australia is an unexplained feature of the data rather than of the method of analysis.

The estimation of penetrance requires a precise statement for each family of the reason that family was ascertained. Failure to correctly specify the reason for the ascertainment will lead to biased estimates of penetrance. The statistical methodology we used to estimate the penetrance of CDKN2A mutations takes a conservative approach by assuming that the reason for the ascertainment of each particular family cannot be defined explicitly. Our approach assumes that the family is ascertained because of the combination of all the affected family members. This approach should lead to an appropriate estimate of penetrance; however, a statistical price is paid in that the confidence intervals of these estimates are broad.

We found no evidence of an effect on CDKN2A mutation penetrance by gender or by whether the CDKN2A mutation altered the predicted p14ARF protein. The former issue—that is, of the potential effect of gender on risk—was suggested by the apparent discrepancy [e.g., *(30)* and Table 1] in gender-specific incidence rates. As for the second issue, we postulated that CDKN2A mutations that had an effect on both p16INK4A and p14ARF might have a more extreme phenotype because such mutations would affect both the retinoblastoma and p53 pathways. However, even though there was not a statistically significant effect of the type of CDKN2A mutation, the analysis suggested a trend toward a higher penetrance among mutation carriers with a mutation that creates both a p16INK4A and a p14ARF coding mutation, with those individuals having an estimated risk 1.8 times that of carriers of a mutation that affects only p16INK4A (Table 3). Our study however, may have had limited statistical power to address this particular issue; larger studies will be needed for a more definitive answer.

We wished to investigate two additional issues relating to inherited melanoma risk, notably, the change in risk in CDKN2A mutation penetrance over time and the contribution of nevi and/or dysplastic nevi to risk. The incidence of melanoma throughout the Western world has been increasing dramatically during the past century, with earlier birth cohorts having lower incidence rates than more recent birth cohorts *(57–59).* Although it would have been interesting to examine the effect of birth cohort on penetrance, we could not do so because of confounding between age, birth cohort, and availability of mutation information. That is, most family members from earlier birth cohorts were deceased at ascertainment of their families and, therefore, unavailable for CDKN2A mutation testing. For ex-

ample, only 397 (38%) of the 1032 family members who were born before 1945 had undergone CDKN2A mutation testing. In contrast, 643 (68%) of the 948 family members who were born since 1945 had undergone CDKN2A testing, and most of them have not yet reached the age where they are at highest risk for melanoma.

We were also not able to examine the effect of nevi on CDKN2A penetrance in this analysis. Whereas the consortium groups had clinically examined many of the family members, the disparity in recording this phenotype precluded comparative analyses. The Melanoma Genetics Consortium is currently developing a standardized scoring system to allow such comparisons.

Despite these limitations, the analyses presented here show that modifying factors of CDKN2A penetrance are to be expected (whether genetic or environmental) and, therefore, that families with many cases of melanoma may share both the CDKN2A mutation as well as other exposures. For this reason, the comparison of penetrance estimates derived from multiple-case families and those derived from population-based studies may well prove to be a fruitful area of investigation. Substantial differences between the two estimates are suggestive of the importance of modifying genetic and/or lifestyle factors. From the evidence produced in this analysis, the estimates of CDKN2A mutation penetrance obtained are, therefore, the appropriate estimates for families with a strong family history of melanoma; they may, however, not be appropriate for individuals without a family history or with a weak family history.

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NOTES

[1]*Editor's note:* SEER is a set of geographically defined, population-based, central cancer registries in the United States, operated by local nonprofit organizations under contract to the National Cancer Institute (NCI). Registry data are submitted electronically without personal identifiers to the NCI on a biannual basis, and the NCI makes the data available to the public for scientific research.

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